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## Influence of Arbuscular Mycorrhiza on Some Physiological Growth Parameters of Pepper

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**Abstract:** Arbuscular Mycorrhiza (AM) is a symbiotic association between plant roots and certain soil fungi. Mycorrhiza establishment is known to modify several aspects of plant physiology such as mineral nutrient composition, hormonal balance, and C allocation patterns. In this study, the effect of the Arbuscular Mycorrhizal fungus *Glomus intraradices* Schenck & Smith on the physiological growth parameters of pepper (*Capsicum annuum* L. cv Cetinel-150) plants was investigated. To explain the physiological growth of these plants, some physiological growth parameters were determined in the shoots and leaves of mycorrhizal (M) and nonmycorrhizal (NM) plants such as the P and dry matter (d.m.) contents, chlorophyll (chl) concentrations (chl a, chl b and chl a + b), and amounts of some reducing sugars (fructose,  $\alpha$  glucose,  $\beta$  glucose), sucrose and total sugar. All parameters increased in M pepper plants by 12%-47% compared with those of the NM plants ( $P \leq 0.01$ ). Furthermore, it was determined that P concentration was positively correlated with all chlorophyll and sugar contents. It is concluded that increased P concentration, because of the mycorrhizal symbioses, positively affects the physiological performance of pepper plants.

**Key Words:** Arbuscular mycorrhizae, *Glomus intraradices*, pepper, chlorophyll, sugar compounds

### Arbusküler Mikorhiza (AM)'nin Biber Bitkisinin Bazı Fizyolojik Gelişim Parametrelerine Etkisi

**Özet:** Arbusküler Mikorhiza, bitki kökleri ile belli baz toprak fungusları arasında kurulan yaygın simbiyotik yaşam şekillerinden biridir. Mikorhiza oluşumu, bitkinin fizyolojik yapısının, mineral madde içeriği, hormonal denge ve karbon kaynaklarının paylaşılması gibi özellikler açısından değişikliğe uğraması olarak da bilinmektedir. Bu çalışmada Arbusküler Mikorhizal (AM) fungus *Glomus intraradices* (Schenck & Smith)'in biber (*Capsicum annuum* L. cv Çetinel) bitkisinin bazı fizyolojik gelişim parametreleri (kurumadde, klorofil, indirgen şeker, toplam şeker, P) üzerine olan etkisi araştırılmıştır. Biber bitkisinin fizyolojik gelişimini açıklayabilmek için mikorhiza aşlanmış (M) ve mikorhiza aşlanmamış (NM) bitkilere ait sap ve yapraklardaki fosfor (P) ve kuru madde miktarı ile klorofil konsantrasyonları (klorofil a, klorofil b ve klorofil a + b), indirgen şeker (fruktoz,  $\alpha$  Glukoz ve  $\beta$  glukoz), sakkaroz ve toplam şeker miktarları gibi bazı fizyolojik gelişim parametreleri belirlenmiştir. Bütün parametreler mikorhiza aşlanmış biber bitkilerinde mikorhiza aşlanmamış olanlara göre daha yüksek bulunmuş ve bu parametrelerin, M bitkilerde NM bitkilere göre % 12 - % 47 oranında arttığı saptanmıştır ( $P \leq 0.01$ ). Bu bağlamda mikorhizal bitkilerdeki P konsantrasyonunun klorofil ve şeker içerikleri ile pozitif bir korelasyon içinde olduğu ancak bu parametrelere ilişkin değişimlerin açıklanmasına yönelik olarak yapılan kısmi korelasyonların istatistiki olarak önemsiz olduğu ortaya konmuştur. Çalışma sonucunda; mikorhizal simbiozis sonucu biber bitkilerinde P içeriğinin arttığı ve bunun da biber bitkilerinin fizyolojik performansını olumlu yönde etkilediği düşünülmektedir.

**Anahtar Sözcükler:** Arbusküler Mikorhiza (AM), *Glomus intraradices*, biber, klorofil, şeker bileşikleri

### Introduction

Arbuscular mycorrhiza (AM) is one of the most widespread Mycorrhizal associations between soil microorganisms and higher plants. The function of all mycorrhizal systems depends on the ability of the fungal symbiont to absorb inorganic and/or organic nutrients available in soil (1). In addition, organic carbon derived from photosynthesis is transferred to these symbionts, which are biotrophic microorganisms, and this substance maintains the development of spores and fruit bodies in

most mycorrhizae types by translocation of the substance to the growing margins of the extraradical mycelium (2). AM has importance due to its great capability to increase plant growth and yield under certain conditions. The major reason for this increase is the ability of plants in association with AM to uptake some nutrients such as phosphorus efficiently (3).

As a result of this symbiotic association between AM fungi and host plants, P content also has an effect on physiological parameters in plants (4,5). One of the

physiological parameters is the increase in photosynthesis. As is known, phosphorous has an important role as an energy carrier during photosynthesis (6). Therefore, AM fungi may function as a metabolic sink causing basipetal mobilization of photosynthates to roots, thus providing a stimulus for greater photosynthetic activity (7). In addition, enhanced levels of cytokinins and gibberellins were found in AM plants. Increases in such hormones, especially cytokinins, could elevate photosynthetic rates by stomal openings influencing ion transport and regulating chlorophyll levels (8,9).

AM fungi, as obligate symbionts, also depend for their growth and activity on the supply of carbon compounds from the photosynthetic partner (10-14). AM symbiosis can cause an important carbohydrate gain in the host plant and up to 20% of total photoassimilate substances can be transferred to the fungal partner (15).

Photosynthetic activity and carbohydrate, which are the photoassimilate substances, are very important in terms of parameters that explain the physiological activity of the plants mentioned above. For this reason, this study aimed to explain the effect of the AM fungus *Glomus intraradices* on the physiological growth of pepper (*Capsicum annuum*) plants using some physiological parameters (or photoassimilate substances) such as sucrose, reducing sugars (fructose, sucrose,  $\alpha$  glucose and  $\beta$  glucose), total sugar and chlorophyll levels (chlorophyll a, chlorophyll b and total chlorophyll) and to expose the relationship between the P content of the plant and these parameters in mycorrhizal pepper plants.

## Materials and Methods

### Cultivation of Plants

Pepper plants were grown in plastic pots (18 x 18 cm) containing a sterilized mixture of soil and sand (1/1, v/v). Table 1 presents the characteristics of the mixture. Fifteen experimental replicates were prepared for each

treatment (each pepper plant was in a separate pot) according to a completely randomized design. Seeds of pepper were surface sterilized with 0.05% sodium hypochloride for 45 min before sowing them into a 5 cm depth of growth media. The plants were grown in a greenhouse under natural photoperiods (23.5/18 °C day/night, 4000-6000 lux light intensity) for 10 weeks during which only distilled water was applied. In addition, twice a week, each pot was supplied with 100 ml of a nutrient solution containing ( $\text{mg l}^{-1}$ ): 720 mg of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 12.2 mg of  $\text{KH}_2\text{PO}_4$ , 295 mg of  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 240 mg of  $\text{KNO}_3$ , 0.75 mg of  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.75 mg of KI, 0.75 mg of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.5 mg of  $\text{H}_3\text{BO}_3$ , 0.001 mg of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 4.3 mg of FeNaEDTA and 0.00017 mg of  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  (23). The symbiotic fungal partner, *Glomus intraradices* (Isolate no. OM/95), was produced in a soil:sand (1/1, v/v) mixture using maize as the host plant. Inoculum of *Glomus intraradices* (30 g), consisting of spores, external mycelium and AMF colonized roots, was laid around the seed. The same amount of sterilized inoculum was laid into the control pots. The percentage of mycorrhizal colonization was estimated by the grid line intersect method (16).

### Harvesting and analyses of plants

At the end of the experiment, plants were harvested 10 weeks after seed sowing. Plant shoots were separated, dried (70 °C 48 h), and weighed. The vanadate-molybdate-yellow procedure was used for P analysis by spectrophotometer (17). The contents of chlorophyll a (Chl a), chlorophyll b (Chl b) chlorophyll a + b (Chl a + b) in leaves were determined by the spectrophotometer according to Smith and Benitez (18). Sugar fractions (sucrose, reducing sugars and total sugar) in shoots and leaves were determined with a 1.65-2 mm diameter glass column bonded with OV-17 chromosorb gas chromatography (modified from 19).

Table 1. The characteristics of the soil mixture used to growt the pepper plant.

pH	O.M. (%)	Soluble Salt (%)	CaCO <sub>3</sub> (%)	total N (%)	P	K	Na	Ca	Mg	Cu	Fe	Zn	Mn
					(mg kg <sup>-1</sup> )								
8.28	1.28	<0.03	6.24	0.056	0.77	200	30	4000	275	0.73	9.66	1.16	15.55

The data were analyzed by linear regression and analysis of variance using SAS, and the means were compared between treatments by the t-test.

## Results

### Root colonization, dry matter and P contents

Mycorrhizal (M) pepper plants were heavily colonized at the rate of 76.7% by *Glomus intraradices* (Table 2). The phosphorus (P) concentration of M plants was higher than that of the nonmycorrhizal (NM) control plants (Table 2) and this parameter was significant ( $P \leq 0.01$ ). The content of dry matter in M plants was also higher than that of NM plants (0.12 g) (Table 2). P and dry matter contents increased in M plants by 35% and 45% compared with those of the NM plants, respectively.

### Chlorophyll content

The chlorophyll content of M pepper plants was higher than that of NM control plants. In particular the amount of chl *a* + *b* increased significantly ( $P \leq 0.01$ ) (Table 2). The contents of chl *a*, chl *b* and chl *a* + *b* increased in M plants by 14%, 12% and 18% compared with those of the NM plants, respectively. The concentration of P was positively correlated with chl *a*, chl

*b* and chl *a* + *b* in M plants although it was not significant (Table 4). Correlation coefficients were determined as  $r = 0.962$ ,  $r = 0.753$  and  $r = 0.879$  in chl *a*, chl *b* and chl *a* + *b*, respectively (Table 4). Partial correlations for all chlorophyll concentrations were also found non significant ( $P > 0.05$ ) (Table 4).

### Sugar content

The amounts of sucrose, reducing sugars (fructose,  $\alpha$  glucose,  $\beta$  glucose) and total sugar, important photosynthetic substances indicating physiological activity, were significantly different between M and NM plants ( $P \leq 0.01$ ) (Table 3). Sugar contents of M and NM pepper plants were 0.57% - 1.37% and 0.13% - 0.75%, respectively (Table 3). Moreover, the amounts of sugar derivatives, sucrose, reducing sugars (fructose,  $\alpha$  glucose,  $\beta$  glucose) and total sugar increased in M plants by 34%, 47%, 43%, 39% and 18%, respectively. On the other hand, the concentration of total P was positively correlated with all sugar derivatives in M plants (Table 4). Correlation coefficients in fructose,  $\alpha$  glucose,  $\beta$  glucose, sucrose and total sugar were  $r = 0.637$ ,  $r = 0.696$ ,  $r = 0.986$ ,  $r = 0.921$  and  $r = 0.308$ , respectively, and for all sugar derivatives partial correlations were not significant (Table 4) ( $P > 0.05$ ).

Table 2. Contents of phosphorus (P), dry matter, Chl *a*, Chl *b* and Chl *a* + *b* in M and NM pepper plants and percentage of AM colonization (AMC). Means  $\pm$  SE, n = 15

Treatments	P (%) **	D.m. (g plant <sup>-1</sup> ) **	Chl <i>a</i> (mg g <sup>-1</sup> ) **	Chl <i>b</i> (mg g <sup>-1</sup> ) **	Chl <i>a</i> + <i>b</i> (mg g <sup>-1</sup> ) **	AMC (%) **
M	0.32 $\pm$ 0.020	0.55 $\pm$ 0.022	0.21 $\pm$ 0.002	0.40 $\pm$ 0.006	0.99 $\pm$ 0.014	76.7
NM	0.09 $\pm$ 0.010	0.12 $\pm$ 0.011	0.15 $\pm$ 0.014	0.33 $\pm$ 0.004	0.54 $\pm$ 0.011	-

Statistically significant differences between M and NM plants are indicated as \*\*  $P \leq 0.01$

Table 3. Amounts of sugars in M and NM pepper plants. Means  $\pm$  SE, n = 15

Treatments	Fructose (%) **	$\alpha$ - Glucose (%) **	$\beta$ - Glucose (%) **	Sucrose (%) **	Total sugar (%) **
M	1.04 $\pm$ 0.089	0.57 $\pm$ 0.021	0.78 $\pm$ 0.012	0.96 $\pm$ 0.017	1.37 $\pm$ 0.095
NM	0.22 $\pm$ 0.031	0.13 $\pm$ 0.013	0.20 $\pm$ 0.023	0.28 $\pm$ 0.012	0.75 $\pm$ 0.013

Statistically significant differences between M and NM plants are indicated as \*\*  $P \leq 0.01$

Table 4. Relationships between contents of total P and amount of chlorophyll a, chlorophyll b, total chlorophyll, reducing sugars (fructose,  $\alpha$ -glucose,  $\beta$ -glucose) sucrose and total sugar in M pepper plants.

	y =	a +	b.x +	c.x <sup>2</sup> +	d.x <sup>3</sup>	R <sup>2</sup>	r
Chlorophyll a <sup>P3</sup>		26.37	-243.42	749.29	-763.02	0.92	0.96 NS
			(-0.96 ns)	(0.96 ns)	(-0.96 ns)		
Chlorophyll b <sup>P3</sup>		-5.47	56.73	-180.93	190.67	0.54	0.73 NS
			(0.59 ns)	(-0.60ns)	(0.61 ns)		
Total chlorophyll <sup>P2</sup>		-0.87	11.34	-16.99	-	0.77	0.87 NS
		(0.85 ns)	(-0.84ns)				
Fructose <sup>P3</sup>		-19.90	166.11	-421.58	339.09	0.40	0.63 NS
		(0.13 ns)	(-0.10ns)	(0.08 ns)			
$\alpha$ -glucose <sup>P3</sup>		-7.21	77.75	-257.25	281.42	0.48	0.69 NS
		(0.27 ns)	(-0.28ns)	(0.30 ns)			
$\beta$ -glucose <sup>P3</sup>		10.77	-98.97	323.42	-348.36	0.97	0.98 NS
		(-0.94 ns)	(0.95 ns)	(-0.95 ns)			
Sucrose <sup>P3</sup>		-24.61	239.52	-737.97	750.35	0.84	0.92 NS
		(0.88 ns)	(-0.88 ns)	(0.88 ns)			
Total Sugar <sup>P3</sup>		36.12	-313.64	936.52	-925.94	0.95	0.30 NS
		(-0.18 ns)	(0.17 ns)	(-0.17 ns)			

<sup>P2</sup>: Polynomial fit (degree 2), <sup>P3</sup>: Polynomial fit (degree 3)

NS: Correlation coefficient is not significant (P > 0.05)

ns: Partial correlation is not significant (P > 0.05)

## Discussion

In this study, the effect of AMF on some of the growth parameters of pepper plants was investigated and the physiological activity of M plants was found to be better than that of NM plants (Tables 2 and 3). One of the most important indicators of physiological activity is the rate of photosynthesis, which is related to the chlorophyll content of plants. In the study, P and the chlorophyll content in M plants were higher than those in NM plants (Table 2) and this also indicated that the photosynthetic rate was improved by AM fungus. Amelioration of the rate of photosynthesis and higher P levels in leaves as a result of AM inoculation were also reported in other studies (4,20,22). In this study, a positive correlation was found between P concentration and chlorophyll content although it was not significant (Table 4). The greater P concentration in M plants compared with that of NM plants of similar size may also have an effect on photosynthesis because the photosynthetic process is known to be positively influenced by the P. Furthermore, since the formation of

mycorrhizae often leads to increases in the leaf area ratio and to leaf hydration, the effect of mycorrhizae on leaf morphology is also probably partly caused by the enhanced P nutrition (6).

Symbiotic interactions in AM associations are based on the exchange of carbohydrates and mineral nutrients between the plant and the fungus. It was demonstrated, using M and NM clover plants of comparable plant size and growth rate and with similar N and P contents, that AM fungal colonization stimulated the rate of photosynthesis sufficiently to compensate for the carbon requirement of the fungus and to eliminate growth reduction of the autotroph (24). In this study, the contents of carbohydrate compounds of M plants such as sucrose, reducing sugars (fructose,  $\alpha$  glucose,  $\beta$  glucose) and total sugar were also usually higher than those of NM plants (Table 2) and a positive correlation was determined between P concentration and all sugar content (Table 3). P also plays the most important role during the breakdown of carbohydrates and/or synthesis of polysaccharides. In particular, P is very effective in the synthesis of starch from glucose (12). As AM fungi

increase the uptake of P, they may also increase the syntheses of carbon compounds (15). Since sugar analyses were performed only in the green parts of the pepper plants in our study, the amount of carbohydrate and/or sugar flow from green parts to the roots was not established. However, it is clear that when the P concentration of plants increases, the photosynthetic rate and its substances also increase and this positively affects the plants (6). Since AM fungi stimulate P uptake and growth in their host plant, they supply carbohydrate compounds for their fungal partner (5). Therefore, it is

seen that photosynthesis activity, increasing as a result of this symbiotic association, has a close relationship with the increase in the function of AM fungi.

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